PERGAMON

Developmental and Comparative Immunology 25 (2001) 639–646

Developmental & Comparative Immunology

www.elsevier.com/locate/devcompimm

Signal transduction pathways activated by engaging immunoglobulin Fc receptors on chicken heterophils

Michael H. Kogut*, Kenneth J. Genovese, David J. Nisbet

USDA-Agricultural Research Service, Southern Plains Agricultural Research Center, 2882 F & B Road, College Station, TX 77845, USA

Received 19 February 2001; revised 16 May 2001; accepted 24 May 2001

Abstract

In the present studies, we initiated experiments to identify the signal transduction factors involved in activating phagocytosis, oxidative burst, and degranulation following the binding of IgG-opsonized SE to Fc receptors on the surface of avian heterophils. Peripheral blood heterophils were isolated and exposed to known inhibitors of signal transduction pathways for either 20 min (chelerythine, genistein, or verapamil) or 120 min (pertussis toxin) at 39°C. The cells were then stimulated for 30 min at 39°C with SE opsonized with IgG purified from SE-immune chickens. Phagocytosis, luminol-dependent chemiluminescence (LDCL), and β-D-glucuronidase release were then evaluated in vitro. The G-protein inhibitor, pertussis toxin, the protein kinase C inhibitor, chelerythine, and the Ca^{++} channel blocker, verapamil, markedly reduced phagocytosis in a dose responsive manner. Genistein, a tyrosine kinase inhibitor, had no effect on the phagocytosis of the opsonized SE. Both pertussis toxin (66–98%) and verapamil (47–76%) had marked inhibitory effect on LDCL. Chelerythine (13–25%) and genistein (5–25%) had far less biologically significant effects on LDCL. Neither chelerythine nor genistein had a significant effect on degranulation. Verapamil (2–28%) and pertussis toxin (25–29%) had a moderate inhibitory effect on degranulation stimulated by IgG-opsonized SE. As was found with complement receptor mediated activation of heterophils, the binding of Fc receptors by the IgG–SE complex activated distinct signaling pathways that regulate the functional activities of avian heterophils. Pertussis toxin-sensitive, Ca^{++}-dependent, G-proteins and protein kinase C-dependent protein phosphorylation play a major role in the phagocytosis of IgG-opsonized SE. Pertussis toxin-sensitive, Ca^{++}-dependent, G-proteins appear to regulate LDCL following Fc receptor binding. The signal transduction inhibitors used in these studies did not affect Fc receptor mediated degranulation by avian heterophils. © 2001 Published by Elsevier Science Ltd.

1. Introduction

Phagocytosis is a multistep process involving particle attachment to the cell membrane and ingestion of the particle. Phagocytosis is triggered by the binding of microbes through either receptors for opsonized particles or nonspecific glycosylated receptors that recognize lectins on target microbes [1–3]. Phagocytic efficiency is dependent on these opsonins bound to the surface of the particle. In serum, the main opsonins are IgG and complement fragments which, when attached to a microbe, can be recognized by specific surface receptors on the phagocytic cell. These specific receptors are the complement receptors that recognize complement fragments and the Fc receptor which recognizes the carboxyl terminus of an antibody

* Corresponding author. Tel.: +1-979-260-3772; fax: +1-979-260-9332.

E-mail address: kogut@ffsru.tamu.edu (M.H. Kogut).

Abbreviations: CHE, chelerythine; GEN, genistein; VER, verapamil; PT, pertussis toxin; LDCL, luminol-dependent chemiluminescence; SE, *Salmonella entertidis*; IgG, immunoglobulin; PMN, polymorphonuclear leukocytes; PKC, protein kinase C; TK, tyrosine kinase.

0145-305X/01/$ - see front matter © 2001 Published by Elsevier Science Ltd.
PII: S0145-305X(01)00026-X

molecule [1,4,5]. The interactions between the surface bound opsonins and the specific receptors initiate a cascade of biochemical reactions in the phagocyte resulting in the activation of defense cell, ingestion of the ligand, and killing of the pathogen [6].

Following binding of a target to one or more surface receptors on polymorphonuclear leukocytes (PMN), a number of biochemical events occur through the generation of intracellular second messengers [7]. The activation of these biochemical events is manifested by the up- or down-regulation of functional activities of the phagocytic cell including increased adhesion, chemotaxis, phagocytosis, degranulation, and production of an oxidative burst. The signal transduction pathways mediating these functional activities are a series of enzyme systems (kinases, phosphatases, phopholipases) and regulatory proteins (G-proteins, channel proteins) associated with the cell membrane which, when activated, generate the production of the second messengers (cAMP, Ca^{++}, diacyglycerol), which regulate the functional activities of the cell [7–10].

To date, a single report on the molecular alterations that accompany the activation of the avian heterophil has been described [11]. Recent evidence has demonstrated that various intracellular signal transduction pathways coordinate the cytoskeletal and biochemical mechanisms involved in the functional stimulation of mammalian neutrophils [4,6,12,13]. Some of the prominent signal transduction factors or second messengers involved in neutrophil activation include protein kinase C, tyrosine kinase(s), intracellular Ca^{++} ions, and G-proteins [8–10,12–14]. Therefore, the objective of the present study was to evaluate the association of signal transduction factors with the enhancement of functional activities of avian heterophils stimulated with an opsonized particulate inflammatory stimulant, i.e. *Salmonella enteritidis* (SE). In these experiments, we compared the effects of different signal transduction inhibitors on the ability of opsonized SE to stimulate phagocytosis, an oxidative burst, and degranulation in vitro.

2. Materials and methods

2.1. Experimental animals

Leghorn chickens (Hy-Line W-36) were obtained on the day-of-hatch from a commercial hatchery (Hy-Line International, Bryan, TX) and randomly placed in electrically heated commercial brooder batteries. Birds were provided water and a balanced unmedicated ration ad libitum. The feed ration contained or exceeded the levels of critical nutrients recommended by the National Research Council [15].

2.2. Isolation of peripheral blood heterophils

Avian heterophils were isolated from the peripheral blood of day-old Leghorn chickens as described previously [16]. Briefly, disodium ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood was mixed with 1% methylcellulose (25 centiposes; Sigma Chemical Co., St Louis, MO) as a 1.5:1 ratio and centrifuged at 25 *g* for 30 min. The serum and buffy coat layers were retained and suspended in Ca^{++}, Mg^{++}-free Hanks' balanced salt solution (HBSS, 1:1; Sigma Chemical Co., St Louis, MO). This suspension was layered over a discontinuous Ficoll-Hypaque (Sigma Chemical Co., St Louis, MO) gradient (specific gravity 1.077 over specific gravity 1.119). The gradient was then centrifuged at 250 *g* for 60 min. After centrifugation, the 1.077/1.119 interfaces and 1.119 band containing the heterophils were collected and washed twice in RPMI 1640 medium (Sigma Chemical Co., S. Louis, MO) and resuspended in fresh RPMI 1640. Cell viability was determined by trypan blue exclusion. The purity of the heterophil suspensions was assessed by microscopic examination of Hema-3 stained (Curtin Mathison Scientific, Dallas, TX) cytospin (Shandon Scientific, Pittsburgh, PA) smears. Heterophil preparations obtained by this method were typically >95% pure and >95% viable. The cell concentration was adjusted to 4×10^6 heterophils/ml and stored on ice until used.

2.3. Bacteria

A poultry isolate of *S. enteritidis* was obtained from the National Veterinary Services Laboratory (NVSL), Ames, IA, and approved by the USDA Animal and Plant Health Inspection Service for use in our facilities. A novobiocin-nalidixic acid (NO-NA) resistant isolate was selected, lyophilized, and stored in 25% sterile glycerol in aliquots of 1×10^9 colony forming units (cfu) at −70°C until used. Medium used to

culture the resistant isolates in experimental studies contained 25 μg/ml novobiocin and 20 μg/ml nalidixic acid to inhibit the growth of other bacteria. Inocula for challenge of *S. enteritidis* were prepared in sterile phosphate-buffered saline (pH 7.2) and adjusted to a concentration of 10^9 cfu/ml using a spectrophotometer with a 625 nm reference wavelength. The viable cell concentration of SE was determined by colony counts on brilliant green agar (BGA) + NO/NA (Oxoid Ltd, Basingstoke, Hampshire, UK) plates.

The overnight culture of SE was killed by adding 0.5% formalin and incubating the culture at 4°C for an additional 24 h. The killed bacteria were washed at least 4 times with cold PBS to remove the formalin, suspended to a concentration of approximately 10^8 bacteria/ml, and then stored at −20°C. The absence of viable SE in the suspension was confirmed by plating on BGA.

2.4. Opsonization of killed Salmonella enteritidis

A hyperimmune serum against the homologous serovar of SE used in the experiments were prepared in 42-week-old layer hens (Hy-Line W-36) [16]. The hyperimmune sera was collected from the blood of these hens and pooled and the pooled sera were octanic acid fractionated and the supernatant submitted to DEAE chromatography. The predominately IgG fractions were pooled and concentrated. The killed SE (10^8 bacteria/ml) were suspended in a subagglutinating concentration of IgG for 30 min at 39°C on a rotary shaker, washed twice with Ca^{++}, Mg^{++}-free HBSS, and stored at 4°C in HBSS (10^8 bacteria/ml) until used.

2.5. Signal transduction inhibitors

Chelerytinine (CHE, a protein kinase C inhibitor), genistein (GEN, a tyrosine kinase inhibitor), verapamil (VER, a Ca^{++} channel blocker), and pertussis toxin (PT, inhibits the regulatory action of G-proteins) were obtained from Sigma Chemical Co. (St Louis, MO). With the exception of PT, all of the inhibitors were dissolved in DMSO and stock solutions of CHE (100 μM), GEN (100 μM), and VER (1000 μM) were stored at 4°C until used. A stock solution of PT (10 μg/ml) was prepared in PBS and stored at 4°C until used. Working concentrations of the inhibitors were prepared in RPMI 1640 tissue culture medium from the stock solutions. The final concentration of DMSO in the experiments was less than 0.5%.

2.6. Phagocytosis assay

Phagocytosis of opsonized SE was determined by using duplicate, sterile 15 ml polypropylene screw-capped centrifuge tubes that contained 2×10^6 heterophils (500 μl), 200 μl of opsonized SE (10^8 cfu/ml), and 300 μl of HBSS. The tubes were incubated at 39°C for 30 min on a rotary shaker to allow for phagocytosis. After each incubation period, the tubes were placed on ice to stop phagocytosis. The cells were washed three times with ice-cold HBSS, centrifuged, and resuspended in 1 ml cold HBSS. Cytospin smears were then prepared from each tube, stained with Hema-3, and examined by light microscopy with the oil immersion objective (100×).

An individual unaware of the groups performed all counts. At least 100 heterophils on each slide were scored for the percentage of heterophils that contained bacteria and for the number of bacteria associated with each heterophil. The results are expressed as the percentage of heterophils that contain bacteria, the average number of bacteria per ingesting heterophil, and the phagocytic index (PI), where PI = the (percentage of heterophils that contain bacteria × the average number of bacteria per ingesting heterophil) × 100.

2.7. Luminol-dependent chemiluminescence assay (LDCL)

The oxidative burst of the purified chicken heterophils was quantified by LDCL adapted from a previously described procedure [17]. Heterophils (4×10^5) and luminol (0.1 M; Sigma Chemical Co., St Louis, MO) in 0.5 ml of RPMI 1640 were placed in Beckman polypropylene scintillation vials. The luminol-dependent chemiluminescence of chicken heterophils was measured in a LKB 1219 series liquid scintillation counter using the tritium channel and the incoincidence mode following stimulation with opsonized SE. LDCL was quantified as counts per minute (c.p.m.) for 1 h. All samples were assayed in replicates of five vials. Results are expressed as peak cpm/10^6 heterophils.

2.8. Degranulation assay

Degranulation was detected by quantifying the amount of β-D-glucuronidase activity in the culture medium following stimulation of the heterophils with opsonized SE. Heterophils (8×10^6) pretreated with or without one of the signal transduction inhibitors, were then incubated with opsonized SE for 1 h on a rocker platform at 39°C in a 5% CO_2 incubator. The reaction was stopped by transferring the tubes containing the cells to an ice bath for 5–10 min. The cells were then centrifuged at 250 *g* for 10 min at 4°C. The supernatants were then removed and used for the assay. Samples (25 μl) of each supernatant were added to quadruplicate wells in a non-treated, black CoStar flat-bottom ELISA plate and incubated with 50 μl of freshly prepared substrate (10 mM 4-methylumbelliferyl-β-D-glucuronide, 0.1% Triton X-100 in 0.1 M sodium acetate buffer) for 4 h at 41°C. The reaction was stopped by adding 200 μl of a stop solution (0.05 M glycine and 5 mM EDTA; pH 10.4). Liberated 4-methylumbelliferone was measured fluorimetrically (excitation wavelength of 355 nm and an emission wavelength of 460 nm) with a *f*max fluorescence microplate reader (Molecular Devices, Sunnyvale, CA). These values were converted to nanomoles of 4-methylumbelliferone generated using a standard curve of known concentrations.

2.9. Experimental design

Heterophils isolated as described above were aliquoted into sterile 15 ml polypropylene screw-capped centrifuge tubes (4×10^6 cells/ml) where they were preincubated with concentrations of the various signal transduction inhibitors, except PT, for 20 min at 39°C. Pertussis toxin required a 2 h preincubation as described previously [11]. Following these preincubations, the heterophils were then stimulated with 10^8 opsonized SE/ml for an additional 30 min at 39°C. After this incubation period, the cells were used in the assays as described above. The anti-coagulated blood from 50 chickens was pooled and the heterophils were isolated from each treatment group as described above. Each heterophil functional assay was conducted four times over a 2-month period with pooled heterophils (heterophils pooled from 50 chickens for each preparation; i.e. 200 total chickens were used as cell donors). At least three replicates were conducted for each heterophil functional assay with the heterophils from each pool of chickens. The data from these four repeated experiments were pooled for presentation and statistical analysis.

2.10. Statistical analysis

The mean and standard error of the mean were calculated for each of the treatment groups in each of the heterophil functional assays. Functional differences between the inhibitor-treated heterophils and non-treated control cells were determined by analysis of variance. Significant differences were further separated using Duncan's multiple range test. The data obtained using heterophils treated with the various signal transduction inhibitors were compared to those obtained with heterophils unexposed to inhibitors (controls).

3. Results

3.1. Phagocytosis

CHE (10 μM), PT (500 and 1000 ng/ml), and VER (10 and 100 μM) had dramatic inhibitory effects on phagocytosis, reducing the percent of heterophils phagocytizing bacteria and the phagocytic index by greater then 65% as compared to the control (Table 1). VER had the most inhibitory effect (60–61%) on the number of SE phagocytized per cell. GEN had no effect on phagocytosis of IgG opsonized SE by chicken heterophils.

3.2. LDCL

The effect of CHE, GEN, VER, and PT on IgG-opsonized SE-induced LDCL is illustrated in Table 2. Both VER (47–76%) and PT (66–98%) significantly inhibited LDCL in a dose responsive manner. Both CHE (25%) and GEN (25%) had inhibitory effects on Fc-mediated LDCL, but neither was as effective as VER or PT.

3.3. Degranulation

None of the four signal transduction inhibitors used

Table 1
The effect of signal transduction inhibitors on the phagocytosis of opsonized *Salmonella enteritidis* by neonatal chicken heterophils. (Data are given as mean ± SD)

Inhibitor	% heterophils with *S. enteritidis*[a] (% decrease)	No. of *S.enteritidis* per heterophil[a] (% decrease)	Phagocytic index[a] (% decrease)
None	83.2 ± 3.1	7.52 ± 1.77	625.7
CHE (μM)			
1	72.2 ± 2.1 (16)	7.78 ± 1.47 (–)	561.7 (11)
10	29.1 ± 4.1** (65)	5.67 ± 0.38* (25)	165.0* (74)
GEN (μM)			
25	84.6 ± 3.2 (–)	7.39 ± 1.57 (–)	625.2 (–)
100	73.7 ± 4.4 (11)	6.80 ± 2.40 (10)	501.2 (20)
VER (μM)			
10	44.9 ± 2.7* (46)	3.01 ± 0.74** (60)	135.1** (78)
100	39.1 ± 1.7* (53)	2.93 ± 0.56** (61)	114.6** (82)
PT (ng/ml)			
500	54.1 ± 4.3* (33)	7.07 ± 0.91 (6)	382.5* (39)
1000	30.8 ± 4.1** (63)	6.99 ± 0.63 (7)	215.3** (66)

[a] Numbers with an asterisk within a single column indicate a significant decrease from control values ($P < 0.05$). Numbers with two asterisks within a single column indicate a significant decrease from control values ($P < 0.01$).

in these studies had a potent effect on Fc-mediated degranulation of chicken heterophils (Table 3). Both VER (2–28%) and PT (25–29%) had a small significant inhibitory effect on the release of β-D-glucuronidase by the heterophils.

4. Discussion

Phagocytosis is the means by which macrophages and polymorphonuclear cells recognize and engulf microbial pathogens. Membrane receptors that parti-

Table 2
The effect of signal transduction inhibitors on opsonized *Salmonella enteritidis*-stimulated luminol-dependent chemiluminescence of neonatal chicken heterophils.(Data are given as mean ± SD)

Inhibitors	LDCL = peak cpm × 10^{6a} (% decrease from control)
None	19.1 ± 1.4
CHE (μM)	
1	16.7 ± 1.1 (13)
10	12.6 ± 0.3* (25)
GEN (μM)	
25	18.1 ± 2.1 (5)
100	14.4 ± 0.7*(25)
VER (μM)	
10	10.2 ± 1.2** (47)
100	4.6 ± 0.3** (76)
PT (ng/ml)	
500	6.5 ± 0.2** (66)
1000	0.4 ± 0.01** (66)

[a] Numbers with an asterisk within a single column indicate a significant decrease from control values ($P < 0.05$). Numbers with two asterisks within a single column indicate a significant decrease from control values ($P < 0.01$).

Table 3
The effect of signal transduction inhibitors on opsonized *Salmonella enteritidis*-stimulated release of β-D-glucuronidase by neonatal chicken heterophils. (Data are given as mean ± SD)

Inhibitors	μM β-D glucuronidase released[a] (% decrease)
None	74.50 ± 1.44
CHE (μM)	
1	78.36 ± 1.18 (–)
10	66.72 ± 0.57 (8)
GEN (μM)	
25	75.76 ± 0.53 (–)
100	63.17 ± 0.32 (16)
VER (μM)	
10	73.27 ± 1.43 (2)
100	53.64 ± 1.77* (28)
PT (ng/ml)	
500	53.14 ± 1.11 (29)
1000	55.79 ± 1.38* (25)

[a] Numbers with an asterisk within a single column indicate a significant decrease from control values ($P < 0.05$). Numbers with two asterisks within a single column indicate a significant decrease from control values ($P < 0.01$).

cipate in this process are the constitutively expressed Fc receptor, which recognizes the Fc portion of antibody molecule, the β_2-integrin receptor (CR3), and receptors that recognize microbial sugar moieties [4,5,18]. Ligation of these receptors activates a variety of cellular responses involved in inflammation and immunity. Activation of Fc receptors stimulates the intracellular signal transduction pathways that initiate phagocytosis, microbial killing, respiratory burst, and degranulation in mammals [6,13]. No such studies have been conducted with avian polymorphonuclear cells. In the present studies, we initiated experiments to identify the signal transduction factors involved in activating phagocytosis, oxidative burst, and degranulation following the binding of IgG-opsonized SE to Fc receptors on the surface of avian heterophils.

Until recently, no information on signal transduction pathways in avian heterophils had been described. However, we have demonstrated that distinct signal transduction pathways differentially regulate the stimulation of the functional activities of avian heterophils via the complement receptor [11]. In our earlier studies, activation of the complement receptor stimulated pertussis toxin-sensitive, Ca^{++}-dependent G-proteins to regulate phagocytosis of serum-opsonized SE while protein kinase C-dependent, tyrosine kinase-dependent protein phosphorylation plays a major role in LDCL, and tyrosine kinase(s)-dependent phosphorylation regulates primary granule release. As shown in the studies described herein, different signal pathways are mediated by the interaction of the Fc receptors on avian heterophils.

PT is a known inhibitor of G-regulatory proteins [19]. As observed in the present studies, treatment of the heterophils with PT resulted in a dramatic inhibition of phagocytosis expressed as percent heterophils phagocytizing SE and phagocytic index (Table 1). Similar findings have been reported with human neutrophils and monocytes and bovine neutrophils [12,20]. Although the precise function of G-regulatory proteins has not been fully described, they appear to be membrane-bound intermediates to cell surface receptors which act as effector enzymes responsible for generating other second messengers (intracellular Ca^{++} and cAMP) [7]. These second messengers are involved in the functional activation of leukocytes [8,9]. The role of intracellular Ca^{++} in Fc receptor-mediated phagocytosis of avian heterophils was also observed by the 55–80% decrease in phagocytosis when the heterophils were treated with the Ca^{++} channel blocker, VER. Furthermore, these results are similar to those found in human neutrophils, where BAPTA, an intracellular Ca^{++} chelator, completely inhibited Fc receptor phagocytosis [21,22]. In contrast with mammalian neutrophils [6,23], we found no effect of the tyrosine kinase inhibitor, GEN, on Fc-mediated heterophil phagocytosis (Table 1). Further research is required to solve this discrepancy between mammalian and avian granulocytic cells. Furthermore, we did observe a dramatic reduction in Fc-mediated phagocytosis following treatment of the heterophils with the protein kinase C inhibitor, CHE (Table 1). Similar findings were reported with both human neutrophils and monocytes [24–26]. Thus, the role of protein kinase C in Fc-mediated phagocytosis may be species and cell type dependent. Therefore, based on the findings from our experiments, the activation of PT-sensitive G-proteins and protein kinase C-dependent protein phosphorylation are critical for the induction of Fc-mediated phagocytosis by avian heterophils.

The generation of an oxidative burst by avian heterophils in response to IgG-ligation of Fc receptors was significantly reduced by treatment with the Ca^{++} channel blocker, VER (10 and 100 μM) and the G-protein inhibitor, PT (500 and 1000 ng/ml) (Table 2). These results are surprising since the oxidative burst in neutrophils appears dependent on the activation of both tyrosine kinases and protein kinase C [27–29]. However, new evidence suggests that phosphatidylinositol 3-kinase (PI3-K) is activated following the cross-linking of Fc receptors [6,24]. Additionally, a specific protein kinase, p38 mitogen-activated protein kinase (p38 MAPK), has been implicated in the phosphorylation of p47-phox [24]. The specific inhibition of p38 MAPK by SB203580 prevented both p47-phox phosphorylation and subsequent superoxide production [24]. Therefore, further research is required using the specific PI3-K inhibitor, wortmannin, and p38 MAPK inhibitor, SB203580, before drawing the conclusions that neither tyrosine kinase nor protein kinase C are involved in the generation of Fc-mediated oxidative burst by avian heterophils.

None of the four signal transduction inhibitors used the present studies had a potent effect on the Fc receptor-mediated degranulation by avian

heterophils (Table 3). These results were in agreement with studies with neutrophils where Fc-receptor-mediated degranulation appears to be a Ca^{++}-independent, phospholipase D-dependent event [30,31].

In conclusion, this is the first report on the intracellular signal pathways initiated by engaging Fc receptors on avian heterophils. The attachment, engulfment, and killing of opsonized microbes are the basis for disease prevention by innate immune defenses. The results from these experiments and from recent studies from our lab have demonstrated that the induction of complement and Fc-receptor-mediated phagocytosis initiates a series of differential intracellular signals [11]. The results from these studies are significant, not only in providing us with a basic understanding of the signals that regulate heterophil functional activity, but also in suggesting ways to maximize the microbicidal activities of heterophils. Based on the results from the experiments described here, pertussis toxin-sensitive, Ca^{++}-dependent G-protein and protein kinase-dependent phosphorylation regulate Fc-mediated phagocytosis. The phagocytic process initiates a pertussis toxin-sensitive, Ca^{++}-dependent G-protein leading to the generation of an oxidative burst. However, further experiments are required to fully define the signals that are activated during the oxidative burst and degranulation.

References

[1] Doer M. Fc receptor biology. Ann Rev Immunol 1997;15:203–34.

[2] Anderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. Ann Rev Immunol 1997;17:593–623.

[3] Franc NC, White K, Ezekowitz RAB. Phagocytosis and development: back to the future. Curr Opin Immunol 1999;11:47–52.

[4] Cohen MS. Molecular events in the activation of human neutrophils for microbial killing. Clin Invest Dis 1994;18:5170–9.

[5] Witthaut R, Farhood A, Smith CW, Jaesche H. Complement and tumor necrosis factor-α contribute to Mac-1 (CD11b/CD18) upregulation and systemic neutrophil activation during endotoxemia in vivo. J Leukoc Biol 1994;55:105–11.

[6] Sanchez-Mejorada G, Rosales C. Signal transduction by immunoglobulin Fc receptors. J Leukoc Biol 1998;63:521–33.

[7] Alberts A, Bray D, Lewis J, Raff M, Roberts K, Watson JD. Molecular biology of the cell. New York: Garland, 1989.

[8] Tauber AI, Karnard AB, Ginnis I. The role of phosphorylation in phagocytic activation. Curr Top Membr Transp 1990;35:469–95.

[9] Divirilio F, Stendel O, Pittet D, Pew PD, Pozzan T. Cytoplasmic calcium in phagocytic activation. Curr Top Membr Transp 1990;35:186–205.

[10] Thelen M, Dewald B, Baggiolini M. Neutrophil signal transduction and activation of the respiratory burst. Physiol Rev 1993;73:797–821.

[11] Kogut MB, Genovese KJ, Lowry VK. Differential activation of signal transduction pathways mediating phagocytosis, oxidative burst, and degranulation by chicken heterophils in response to stimulation with opsonized *Salmonella enteritidis*. Inflammation 2001;25:7–15.

[12] Yu PW, Czuprynski CJ. Regulation of luminol-dependent chemiluminescence and degranulation by bovine neutrophils stimulated with opsonized zymosan. Vet Immunol Immunopathol 1996;50:29–42.

[13] Edwards SW. Cell signaling by integrins and immunoglobulin receptors in primed neutrophils. Trends Biochem Sci 1995;20:362–7.

[14] Dewald B, Thelen M, Wyman MP, Baggiolini M. Staurosporin inhibits the respiratory burst and induces exocytosis in human neutrophils. Biochem J 1989;264:878–84.

[15] National Research Council. Nutrient requirements of poultry, 8th ed. Washington DC: National Academy Press, 1984.

[16] Kogut MB, McGruder ED, Hargis BM, Corrier DE, DeLoach JR. In vivo activation of heterophil function in chickens following injection with *Salmonella enteritidis*-immune lymphokines. J Leukoc Biol 1995;57:56–62.

[17] Kogut MH, Holtzapple C, Lowry VK, Genovese KJ, Stanker LH. Functional responses of neonatal chicken and turkey heterophils following stimulation by inflammatory agonists. Am J Vet Res 1998;59:1401–8.

[18] deHaas M, Vossebeld PJM, von dem Borne AEG, Roos D. Fc receptors of phagocytes. J Lab Clin Med 1995;126:330–41.

[19] Moss J, Vaughn M. ADP-ribosylation of guanyl nucleotide-binding regulatory proteins by bacterial toxins. Adv Enzymol Relat Areas Mol Biol 1988;66:303–79.

[20] Cockcroft S. G-protein regulated phospholipases C, D, and A_2-mediated signaling in neutrophils. Biochim Biophys Acta 1992;113:135–60.

[21] Rosales C, Brown EJ. Calcium channel blockers nifedipine and diltiazen inhibit Ca^{2+} release from intracellular stores in neutrophils. J Biol Chem 1992;267:1443–8.

[22] Kobayashi K, Takahashi K, Nagasawa S. The role of tyrosine phosphorylation and Ca^{2+} accumulation in Fcγ-receptor-mediated phagocytosis of human neutrophils. J Biochem 1995;117:1156–61.

[23] Indik ZK, Park JG, Pan XQ, Schreiber AD. Induction of phagocytosis by a protein tyrosine kinase. Blood 1995;85:1175–80.

[24] Yamamori T, Inanami O, Nagahata H, Cui YD, Kuwabasa M. Roles of p38 MAPK, PKC, and PI3-K in signaling pathways of NADPH oxidase activation and phagocytosis in bovine polymorphonuclear leukocytes. FEBS Lett 2000;467:253–8.

[25] Fallman M, Gullberg M, Hellberg C, Andersson T. Complement receptor-mediated phagocytosis is associated with accumulation of phosphatidylcholine-derived diglycerol in human neutrophils. Involvement of phospholipase D and direct evidence for a positive feedack signal of protein kinase. J Biol Chem 1992;267:2656–63.

[26] Zheleznyak A, Brown EJ. Immunoglobulin-mediated phagocytosis by human monocytes requires protein kinase C activation. Evidence for protein kinase C translocation to phagosomes. J Biol Chem 1992;267:12042–8.

[27] Brumell JH, Burkhardt AL, Bolen JB, Grinstein S. Endogenous reactive oxygen intermediates activate tyrosine kinases in human neutrophils. J Biol Chem 1996;271:1455–61.

[28] Falkow L, Chan CK, Grinstein S, Downey GP. Regulation of tyrosine phosphorylation in neutrophils by the NADPH oxidase. J Biol Chem 1993;268:17131–7.

[29] Fernandez R, Suchard SJ. Syk activation is required for spreading and H_2O_2 release in adherent human neutrophils. J Immunol 1998;160:5154–62.

[30] Gewirtz A, Simmons ER, Phospholipase D. mediates Fc receptor activation of neutrophils and provides signaling specificity between chemtactic and phagocytic signaling pathways. J Leukoc Biol 1997;61:522–8.

[31] Seetoo KF, Schonhorn JE, Gewirtz AT, Zhou MJ, McMenanin ME, Delva L, Simmons ER. A cytoplasmic calcium transient is not necessary for degranulation of oxidative burst in immune complex-stimulated neutrophils. J Leukoc Biol 1997;62:329–40.